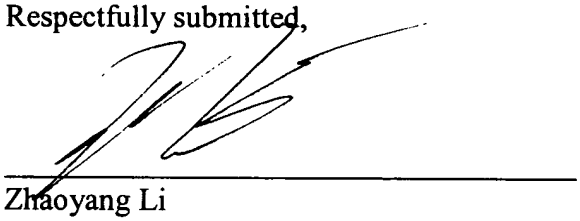
Remarks

Applicants have attached a marked-up version of the amended specification pages pursuant to 37 C.F.R. § 1.121(b)(1)(iii), and a clean copy of the specification pages pursuant to 37 C.F.R. § 1.121(b)(1)(ii).

Respectfully submitted,



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Marked-up Copy of Amended Specification Pages
Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

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Figure 5B is the map of inserts in the plant transformation vector for leaf-specific expression of *phaG, alkK* and *phaC* for the accumulation of polymer in the cytosol of leaves.

Figure 5C is the map of inserts in the plant transformation vector for leaf-specific expression of *phaG, alkK* and *phaC* for the accumulation of polymer in the peroxisomes of leaves.

Figure 6A is the map of inserts in the plant transformation vector for seed-specific expression of *phaG, alkK* and *phaC* for the accumulation of polymer in the plastids of seeds.

Figure 6B is the map of inserts in the plant transformation vector for seed-specific expression of *phaG, alkK* and *phaC* for the accumulation of polymer in the cytosol of seeds.

Figure 6C is the map of inserts in the plant transformation vector for seed-specific expression of *phaG, alkK* and *phaC* for the accumulation of polymer in the peroxisomes of seeds.

Detailed Description Of Invention

The inability of *E. coli* to form medium chain length PHAs from glucose when expressing PhaG and PhaC suggests that an additional enzyme activity may be required when this pathway is engineered into non-native PHA producers that are not Pseudomonads. U.S. Patent No. 5,750,848 describes screening methods to isolate an enzyme or combination of enzymes that allow conversion of 3-hydroxy acyl ACPs to 3-hydroxy acyl CoAs in PHA negative bacteria, but such enzymes [have not been described in the literature nor] <u>are not described</u> in the patent. Klinke et al. (Klinke, S., Ren, Q., Witholt, B., Kessler, B. Appl. Environ. Microbiol. 1999, 65, 540-548) have demonstrated PHA production in *E. coli* upon coexpression of a thioesterase and a PHA synthase. Since the thioesterase employed [in this study] <u>by Klinke et al.</u> does not convert 3-hydroxy acyl ACPs to 3-hydroxy fatty acids but instead converts acyl ACPs to fatty acids, the host cell's native β-oxidation enzymes are required to form the 3-hydroxy acyl CoAs for PHA formation. This strategy is therefore limited in

The sequence of *alk*K in the plant expression construct pUC-C4PPDK.TS.AlkK is shown in SEQ ID NO:13. The sequences "tctaga" (SEQ ID NO:14) and "ggtacc" (SEQ ID NO:15) are XbaI and KpnI restriction sites, respectively, introduced for cloning purposes. [The start codon "ATG" and stop codon "TGA" are indicated in capital letters. The two sequence discrepancies observed in the PCR product are indicated in bold capital italic letters.] Plasmid pUC-C4PPDK.TS.AlkK was used as a starting plasmid to create other plant expression constructs containing *alkK*.

For PHA production in the chloroplasts of leaves, plasmid pCambia-C4PPDK.TS.PhaG.TS.AlkK.TS.$PhaC_{P.o.}$ (Figure 5A) was designed. Plasmid pCambia-C4PPDK.TS.PhaG.TS.AlkK.TS.$PhaC_{P.o.}$ contains the 35S-C4PPDK promoter, the pea rubisco chloroplast targeting signal including DNA encoding the N-terminal 24 amino acids of the pea rubisco protein, a fragment encoding PhaG, a Nos termination sequence, the 35S-C4PPDK promoter, the pea rubisco chloroplast targeting signal including DNA encoding the N-terminal 24 amino acids of the pea rubisco protein, a fragment encoding AlkK, a Nos termination sequence, the 35S-C4PPDK promoter, the pea rubisco chloroplast targeting signal including DNA encoding the N-terminal 24 amino acids of the pea rubisco protein, a fragment encoding PhaC from *Pseudomonas oleovorans* and a Nos termination sequence.

Plasmid pCambia-C4PPDK.TS.PhaG.TS.AlkK.TS.$PhaC_{P.o.}$ can be transformed into *Arabidopsis*, as previously described in Example 5, or Tobacco, as described in the following procedure. In a laminar flowhood under aseptic conditions, leaves from a tobacco plant are sterilized for 15 minutes in a one liter beaker containing a solution of 10% bleach and 0.1% Tween 20. The sterilized leaves are washed in one liter of water for 10 minutes, the water is decanted, and the washing step is repeated two additional times. The intact part of the leaves are cut in small pieces with a scalpel, avoiding any injured areas of the leaves. An aliquot (20 mL) of MS-suspension is mixed with 5 mL of an overnight culture of *Agrobacterium* strain GV3101/pMP90 (Konz, C. & Schell,

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Plasmid pCambia-C4PPDK.TS.PhaG.TS.AlkK.TS.PhaC$_{P.o.}$ can be transformed into *Arabidopsis*, as previously described in Example 5, or Tobacco, as described in the following procedure. In a laminar flowhood under aseptic conditions, leaves from a tobacco plant are sterilized for 15 minutes in a one liter beaker containing a solution of 10% bleach and 0.1% Tween 20. The sterilized leaves are washed in one liter of water for 10 minutes, the water is decanted, and the washing step is repeated two additional times. The intact part of the leaves are cut in small pieces with a scalpel, avoiding any injured areas of the leaves. An aliquot (20 mL) of MS-suspension is mixed with 5 mL of an overnight culture of *Agrobacterium* strain GV3101/pMP90 (Konz, C. & Schell, J. Mol. Gen. Genet. 1986, 204, 383-396) carrying the construct to be transformed [MS-suspension contains (per L) 4.3 g MS salts, 1 mL of B5